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14. ABSTRACT

The chemical properties and high density of depleted uranium (DU) render the metal well suited for military purposes, but knowledge of DU neurotoxicity and its treatment is lacking. This project is designed to test the hypothesis that *long-term administration of an anti-oxidant agent and/or an NMDA receptor antagonist will reduce neurotoxicity resulting from chronic exposure to DU.* Major scientific findings of the project included: 1) absence of depolarization-evoked increases in hippocampal extracellular glutamate in DU groups after a 9-month period of chronic exposure; 2) modest DU-induced increases in cerebellar lipid peroxidation in the absence of changes in cerebellar/striatal redox enzymes; 3) substantial DU-related increases in access to the NMDA receptor channel in frontal cortex; and 4) failure of memantine and/or riluzole to diminish or eliminate DU-induced neurotoxicity in a therapeutically useful manner. Thus, there is no evidence suggesting that these drugs offer clinical value as new therapeutic approaches to treat DU neurotoxicity.

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INTRODUCTION

The chemical properties and high density of depleted uranium (DU) render the metal well suited for military purposes. The U. S. Army utilizes DU for tank armor and in munitions, deployed such weapons in the first Gulf War, and is currently deploying them in Afghanistan. However, knowledge of DU neurotoxicity and its treatment is lacking despite reports of exposed cohorts exhibiting neurocognitive dysfunction (1). Research in chronically exposed rats has reported alterations in hippocampal synaptic transmission, suggesting DU-induced decreases in neuronal excitability (2). This project examined potential treatment options to address neurotoxicity from chronic DU exposure. On the basis of previous observations the underlying effects of DU neurotoxicity were proposed to be cellular oxidative stress and the consequent increased production of reactive oxygen species, leading to decreased glutamate uptake and increased synaptic glutamate concentrations in conjunction with NMDA receptor up-regulation. Uranium-induced oxidative stress has previously been reported in rat kidney, testis, and lung (3-4). Studies were designed to identify various biochemical markers of metal-induced oxidative stress in CNS tissue, and in combination with enhanced extracellular glutamate and NMDA receptor activity would provide three components of DU neurotoxicity for assessment of therapeutic efficacy. *It was hypothesized that long-term administration of an anti-excitotoxic agent and/or an NMDA receptor antagonist would reduce DU neurotoxicity.* It was hoped that these studies would provide critical information on which to base new treatments for exposed Gulf War veterans.

BODY

The findings of this investigation will be presented using the Statement of Work as a framework. A description of these efforts and the resulting completion of each objective are provided below.

Task 1 concerned efforts to demonstrate the efficacy of chronically administered drug therapies to reverse DU-induced elevations in extracellular glutamate in superfused hippocampal slices from chronically exposed animals. The project included a control group (tantalum pellets instead of DU) and low (300 mg load) and high dose (600 mg load) DU exposure conditions, but also utilized a vehicle and three drug-treated groups (memantine or riluzole or a combination) for each exposure level. This design resulted in a 3 exposure level × 4 drug condition matrix with ~8 animals/cell, thus maximizing the ability to discriminate the actions of the therapeutic agents on the proposed measures. Drugs were administered via osmotic minipumps (Alzet) surgically inserted subcutaneously. Adult male Sprague-Dawley rats were implanted intramuscularly with DU pellets at 70-80 days of age; beginning at 3-4 months of age they were placed on food restriction so that their body weight consistently remained in the range of 500-550 grams. After 7 months exposure 28-day osmotic minipumps were implanted and replaced once to cover the period up to 9 months when exposure was terminated and testing conducted. This was an appropriate interval for drug administration as this regimen simulated DU exposure during the first Gulf War and potential treatments applied long after exposure was initiated. The minipumps were filled with drug solutions of 30 mg/ml memantine (3.6 mg/kg/day dose) and/or 10 mg/ml riluzole (1.2 mg/kg/day dose). Besides its potential usefulness as an uncompetitive NMDA receptor antagonist, memantine also has been reported to have neuroprotectant value via induction of brain-derived neurotrophic factor (BDNF) and its receptor (5-7), making the drug of particular interest for this project. Blood samples were collected from sufficient animals prior to sacrifice to establish the plasma drug levels attained and validate the drug administration protocols. Table 1 lists the plasma drug levels achieved over a period of 7-8 weeks with this dosing regimen.

Plasma Drug Levels, ng/ml		
Group	**MEM**	**RIL**
Vehicle	**- 0**	**-- 0**
Memantine	**28.1 ± 3.4**	**--**
Riluzole	**--**	**41.1 ± 3.0**
Memantine & Riluzole	**17.2 ± 1.2**	**24.5 ± 2.3**
Values are mean ± SEM, N = 8-9. Blood sampled from jugular vein 7-8 weeks after drugs instituted. MEM = memantine; RIL = riluzole.		

Evidence from previous work indicated that the effects of chronic exposure to DU on depolarization-induced hippocampal extracellular glutamate are a combination of acute UO_2^{+2}-dependent inhibition of release (8) and an opposing slowly developing increase in extracellular transmitter (9), perhaps due to metal-induced oxidative stress leading to mitochondrial and glutamate transporter dysfunction. An additional component of the observed neurotoxic response was a substantial up-regulation of access to the NMDA receptor channel. Data from the study addressing Task 1 are shown in Figures 1-3. The results display depolarization-induced glutamate release from K^+-stimulated hippocampal slices after

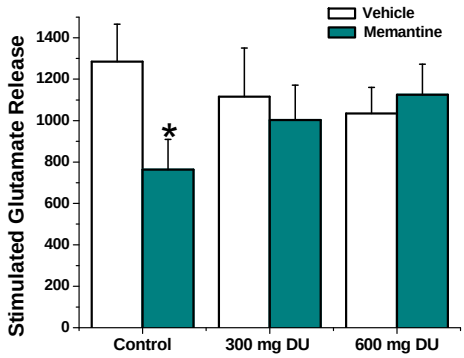

Figure 1. Evoked glutamate release from K^+-stimulated hippocampal slices after exposure to DU for 9 months and continual administration of memantine for months 8-9 via osmotic minipumps. Values are expressed as mean ± SEM (N = 7/group) of the area under the curve normalized to 100 and summed across the peak response intervals. * p <0.05 compared to the paired group receiving vehicle only in the minipumps.

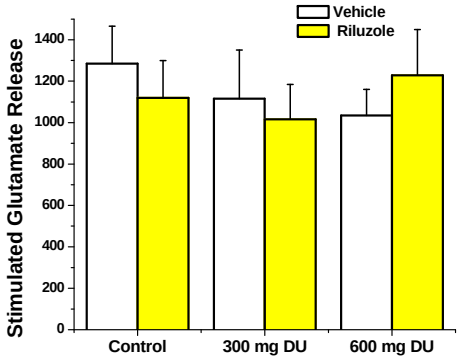

Figure 2. Evoked glutamate release from K^+-stimulated hippocampal slices after exposure to DU for 9 months and continual administration of riluzole for months 8-9 via osmotic minipumps. Values are expressed as mean ± SEM (N = 6-7/group) of the area under the curve normalized to 100 and summed across the peak response intervals.

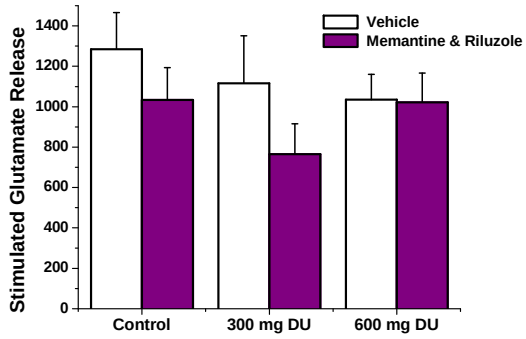

Figure 3. Evoked glutamate release from K^+-stimulated hippocampal slices after exposure to DU for 9 months and continual administration of memantine and riluzole for months 8-9 via osmotic minipumps. Values are expressed as mean ± SEM (N = 6-7/group) of the area under the curve normalized to 100 and summed across the peak response intervals.

exposure to DU for 9 months in conjunction with continual subcutaneous administration of memantine (Figure 1), riluzole (Figure 2), or the combination (Figure 3) for months 8-9 via osmotic minipumps. Analogous measures of extracellular GABA could not be obtained due to an interfering chromatographic peak emanating from the osmotic minipumps. Previous work has shown that extracellular GABA responds to DU exposure in a qualitatively similar manner to that of glutamate (9).

An additional aspect of Task 1 was to obtain a direct measure of glutamate uptake in transverse 400 µm thick sensorimotor cortical slices from identical chronically DU-exposed and drug treated animals to assess whether glutamate transporter integrity might play a role in the changes observed in extracellular glutamate concentrations. Slice uptake was measured in the presence of three extracellular concentrations of the transmitter in the incubation medium, each containing tracer amounts of ^{3}H-glutamate. Replicate slices from each animal were tested in the presence and absence of Na^{+} in the assay medium with choline chloride used to define Na^{+}-independent uptake. As shown in Figure 4, there were no significant effects of chronic DU exposure on glutamate transporter function at any of the applied transmitter concentrations. Analogous data in slices from animals receiving memantine, riluzole, or their combination did not uncover statistically significant effects and are not shown.

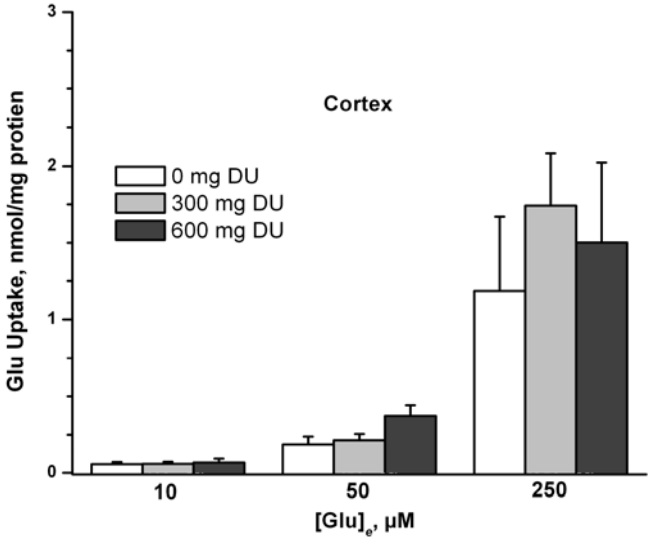

Figure 4. Glutamate uptake in cortical slices from chronically DU-exposed animals. Uptake was determined at each of three transmitter concentrations in the incubation medium using ^{3}H-glutamate in the presence and absence of Na^{+} in the assay buffer. No changes were observed in glutamate uptake. Values are expressed as mean ± SEM of the difference between Na^{+}-dependent and –independent uptake with N = 5-8/group for each measure.

In addition, independent control and DU groups were generated that did not receive minipumps containing vehicle, and thus more closely simulated the exposure of study groups in the earlier project. Data on stimulated glutamate release in this small cohort are shown on the left side of Figure 5. As a result of the data in Figures 4 and 5 two observations are apparent. First, the enhancement of evoked extracellular glutamate concentrations found after 14-17 months of DU exposure in earlier work was not discriminated after the shorter 9 months of exposure (Figure 5), suggesting that longer periods of administration are necessary for the glutamate transporter dysfunction to become evident as well as the dominant effect over the opposing UO_2^{+2}-dependent inhibition of release. Second, memantine significantly diminished evoked extracellular glutamate in control vehicle-treated animals (Figure 1), but had no effect on groups chronically exposed to DU. Administration of riluzole and the memantine/riluzole combination did not alter glutamate responses to high K^{+} in any groups.

The bases for the attenuation of stimulated glutamate release by memantine in control animals, and the

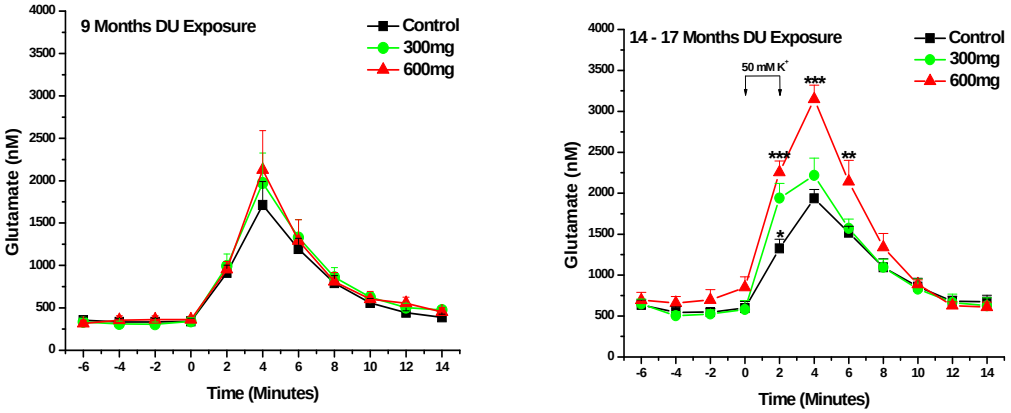

Figure 5. Time course of glutamate concentration in response to superfusion with 50 mM K^+ across transverse hippocampal slices in 10 mM HEPES-sucrose buffer (pH 7.4). The stimulus-evoked increase in endogenous glutamate was significantly enhanced by exposure to 300 or 600 mg implanted DU over 14-17 months (*Right*), but not at 9 months (*Left*). Values are expressed as mean ± SEM based on N = 8/group with sample determinations conducted in triplicate. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ relative to the glutamate concentration in control animals at the same time point.

absence of this effect in the DU-exposed groups requires further investigation. Since memantine is an antagonist at NMDA receptors, the DU-induced receptor up-regulation may result in a smaller proportion of inhibited NMDA receptors in the exposed than in the control groups, and thereby cause reduced drug effectiveness in DU animals. However, memantine also exhibits other pharmacological actions that must be considered, such as inhibiting nicotinic receptors (10) and enhancing BDNF function (e.g., 7).

It is possible that the doses of memantine/riluzole utilized were not adequate to produce more consistent effects in the control and DU groups. If this were true, another route of administration should be chosen, since the concentrations of drug applied through the osmotic minipumps approached their solubility limits. A longer duration of drug administration may also have been more effective in countering DU neurotoxicity, but such a regimen would have less relevance to the Gulf War cohort of exposed soldiers who currently remain without focused treatment.

Task 2 consisted of determination of DU concentrations in brain tissue of chronically exposed animals at durations corresponding to the beginning (7 months) and end (9 months) of the drug therapies. Characterization of the protocol in this Task was designed to provide context to the experimental findings generated under the other Tasks. The determination of hippocampal uranium levels was to be performed by inductively coupled plasma-mass spectrometry (ICP-MS) analysis by a commercial laboratory. This methodology has proven more sensitive and reliable for this sample matrix than alternative approaches. The DU used in this project consisted of 30 mg pellets (1 mm diameter × 2 mm length) obtained from Aerojet Ordnance Tennessee (Jonesborough, TN), and were sterilized prior to use. Ten pellets were implanted in the gastrocnemius muscle of each thigh of 70-80 day old male rats. The design included three exposure groups: a high dose group in which all pellets were DU (600 mg load), a low dose group receiving 10 pellets of DU (300 mg load), and a control group which received 20 tantalum pellets (0 mg load). The low dose group also received 10 pellets of tantalum. Tantalum is an essentially inert heavy metal widely used in medical prostheses. The group size (N =

6) was sufficient to characterize the exposure protocol and provide general measures of metal uptake. All tissue samples for these analyses have been harvested and remain available for analytical determination. However, the DU analyses to complete Task 2 (cost of ~$10K) have not been performed as funds have not been identified to cover the expense. Because of the thorough characterization of the exposure regimen in earlier work (9, see Appendix II) and the cost of other unforeseen expenses (see Table 1 and below), these analyses have not been executed.

In order to maximize the efficiency of use of the last cohort of animals Tasks 3 and 4 were performed on brain tissue harvested from this same group of rats. Task 3 concerned assessments of biochemical markers of DU-induced oxidative stress in brain tissue and the ability of drug therapies to reverse the changes in these measures. Brains were harvested and cerebellum and striatum dissected to enable these determinations.

Glutathione peroxidase activity was measured indirectly in striatal samples by a coupled reaction with glutathione reductase in which oxidized glutathione – produced upon reduction of hydroperoxide by glutathione peroxidase – is recycled to its reduced state by the reductase and NADPH (11). The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance. Catalase activity was determined in one half of symmetrically divided cerebellar samples based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 (12-13). The formaldehyde produced is measured colorimetrically with a triazole chromogen. As shown in Figure 6, there were no significant effects of chronic DU exposure on either enzyme activity. Analogous data in these brain regions from animals receiving memantine, riluzole, or their combination did not uncover statistically significant effects and are not shown.

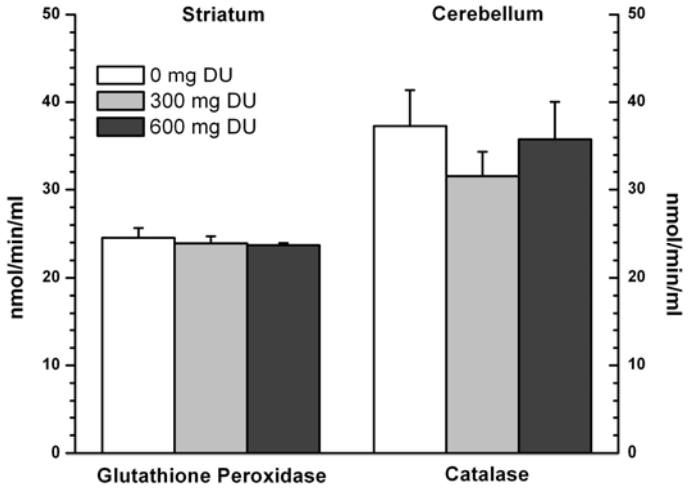

Figure 6. Striatal glutathione peroxidase and cerebellar catalase activity in chronically DU-exposed animals receiving only vehicle via osmotic minipump for two months. There are no changes in enzyme activity that would suggest an increase in oxidative stress. Values are expressed as mean ± SEM based on N = 6-8/group with sample determinations conducted in triplicate.

It was recently determined in a multi-investigator NIH study that quantification of F_2-isoprostanes represented the most accurate method to assess lipid peroxidation status *in vivo* (14). F_2-isoprostanes are prostaglandin-like compounds produced by a non-cyclooxygenase free radical-catalyzed mechanism involving the peroxidation of the polyunsaturated fatty acid arachidonic acid. 8-Isoprostane has been proposed as a marker of antioxidant deficiency and oxidative stress. The assay is based on the competition between 8-isoprostane and an 8-isoprostane-acetylcholinesterase conjugate for a limited number of compound-specific rabbit antiserum binding sites. Because the concentration

of the conjugate tracer is held constant while the sample concentration of 8-isoprostane varies, the amount of tracer able to bind to the antiserum is inversely proportional to the sample concentration of 8-isoprostane.

As shown in Figure 7 (*Left*), in animals chronically exposed but receiving no drugs the percentage of the 8-isoprostane tracer bound was significantly reduced in cerebellum in the DU groups compared to control animals ($F_{2,35}$ = 20.00, $p < 0.001$). This corresponded to a small but significant increase in sample 8-isoprostane levels in vehicle DU groups ($F_{1,35}$ = 246.42, $p < 0.001$) shown in the *Right* graph. Moreover, groups receiving memantine exhibited a significantly larger proportion of binding of the tracer (*Left*, $F_{2,35}$ = 16.98, $p < 0.001$) and significantly lower concentrations of 8-isoprostanes (*Right*, $F_{1,35}$ = 439.47, $p < 0.001$) than the vehicle groups, indicative of the drug's hypothesized neuroprotective actions. But the effect of DU exposure was not eliminated. Because of the effectiveness of memantine observed in other Tasks (see Figures 1 and 8) and the high costs of the reagents to perform the 8-isoprostane assays, other drug groups were not tested.

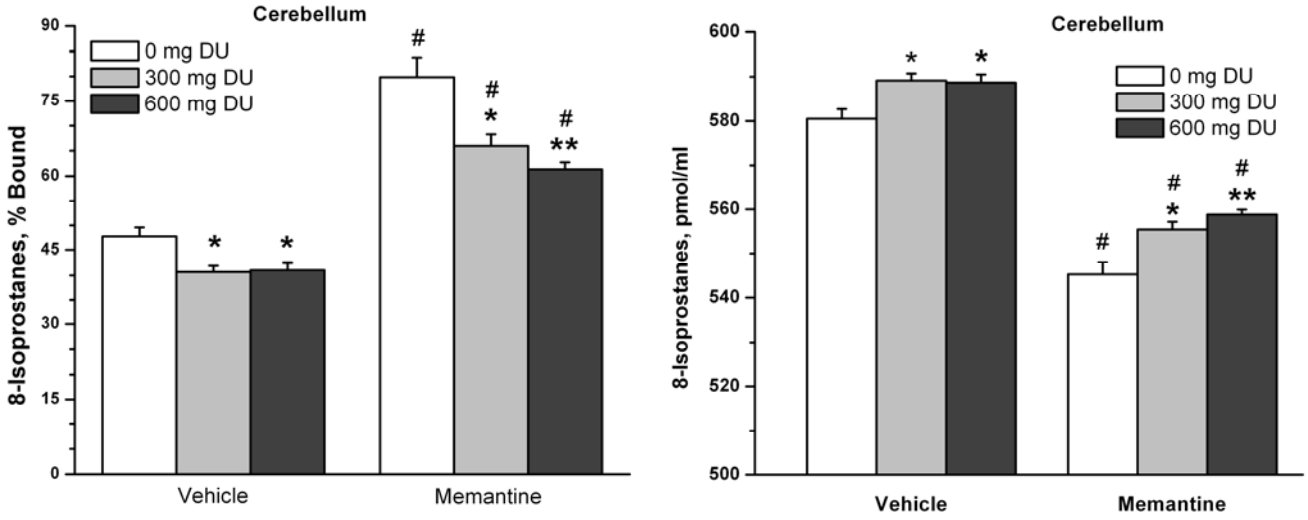

Figure 7. Percent bound of 8-isoprostane tracer from competition ELISA (*Left*) and resulting sample 8-isoprostane concentrations (*Right*) in cerebellum in control and DU-exposed animals receiving either vehicle or memantine. Both measures indicated the presence of modest increases in lipid peroxidation as a result of DU exposure; memantine exhibited some neuroprotection but did not reverse the effects of DU. Values are expressed as mean ± SEM with N = 6-8/group with sample determinations conducted in triplicate. $^{**}p < 0.01$; $^{*}p < 0.05$ compared to the corresponding vehicle or memantine control group. $^{\#}p- < 0.001$ compared to the corresponding exposure group receiving vehicle.

Thus, completion of Task 3 indicated that the activities of two antioxidant enzymes in striatum and cerebellum were not altered by 300 or 600 mg of indwelling DU exposure for a 9-month period, but that a modest amount of DU-induced lipid peroxidation was present in cerebellum. There was some evidence therefore that DU produced oxidative stress in brain tissue as has been reported in other organs (3-4).

Task 4 quantified DU-induced elevations in NMDA receptor binding density and the ability of drug therapies to reverse these measures in tissue membrane preparations using radioligand binding. Brains were harvested from the last cohort of exposed animals (see Task 3 above) and frontal cortex dissected and stored at -80°C. Radioligand binding assays were performed with 8 concentrations of unlabelled-MK-801 (2.5 – 1000 nM) to displace a constant concentration of ^{3}H-MK-801 (25 nM) in independent

membrane preparations from each animal. MK-801 binding essentially measures accessibility to the cell membrane NMDA receptor-operated cation channel.

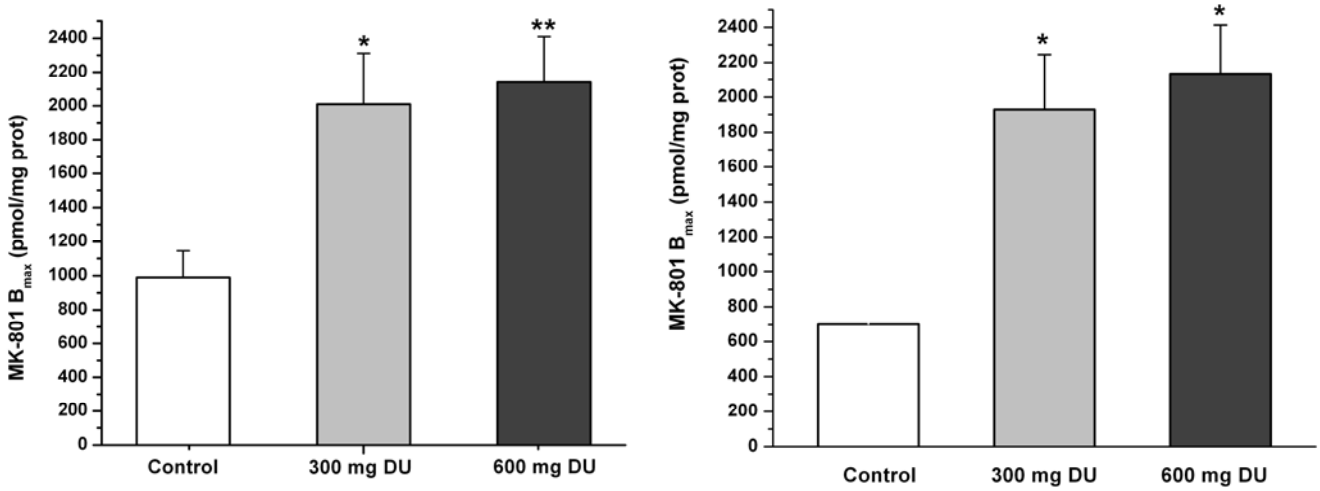

Figure 8. Density of hippocampal *(Left)* and sensorimotor cortical *(Right)* NMDA receptors as measured by displacement of ^{3}H-MK-801 binding in animals as a function of the amount of implanted DU pellets over 16-17 months exposure from earlier studies, shown for comparison with **Figure 9** below. Values are mean ± SEM with each mean based on 6-7 (hippocampal) or 3-4 (cortical) Scatchard analyses conducted in triplicate with each analysis based on tissue pooled from two animals. $^{**}p < 0.01$; $^{*}p < 0.05$ compared to Control value.

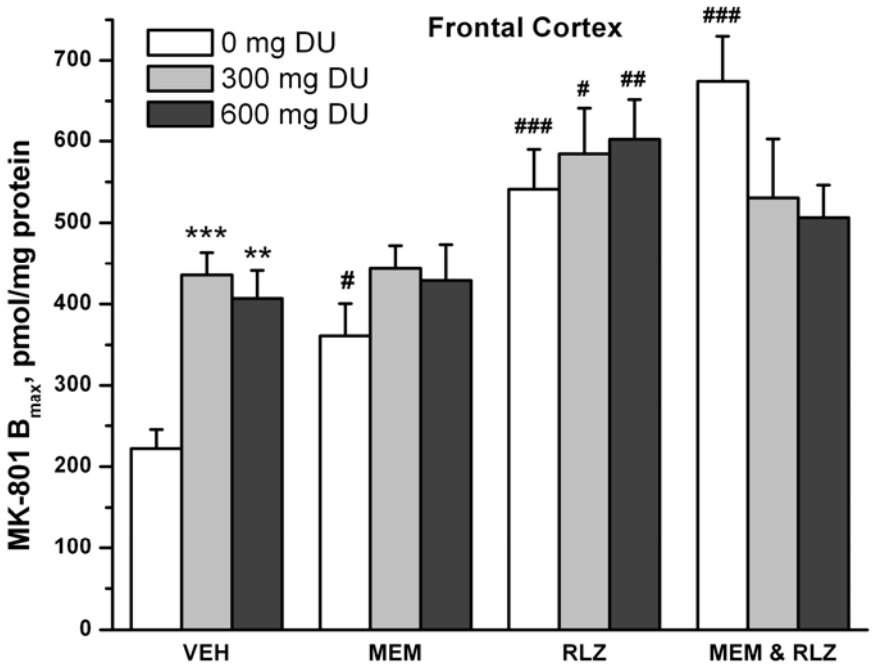

Figure 9. Density of frontal cortical NMDA receptors as measured by displacement of ^{3}H-MK-801 binding in animals receiving memantine (MEM), riluzole (RLZ), or the combination (MEM + RLZ) during the last 2 months of chronic DU exposure. Both MEM and RLZ eliminated the up-regulation of receptor density seen in animals receiving vehicle only (VEH), but did so by increasing receptor binding in control animals. Values are expressed as mean ± SE with N = 4-8/group and amounts bound averaged across all animals in a group at each unlabeled MK-801 concentration. $^{**}p < 0.01$; $^{***}p < 0.001$ compared to the vehicle-treated control group. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ compared to the corresponding vehicle-treated group.

As shown in Figure 9, chronic DU exposure resulted in a striking up-regulation of access to the NMDA receptor channel in animals not receiving drug treatment of 96% and 83% in the 300 and 600 mg dose groups, respectively, compared to control values. No changes were found in the binding affinity constants K_D observed across DU-exposed and drug-treated groups (data not shown). The magnitude of these DU-induced changes are in general agreement with previous observations of 100-200% increases in NMDA receptor binding in hippocampus and sensorimotor cortex (see Figure 8 from earlier work) at the same exposure levels for longer durations (15). The up-regulation of NMDA receptor access is in contrast to reports of decreased hippocampal neuronal excitability after long-term DU exposure (2). However, since MK-801 binds to a site within the ion channel, it is not possible to distinguish whether the enhanced receptor density is due to increased expression of receptor protein or to a DU-induced conformational change in the receptor that confers greater access to the channel site for this ligand. Increases in NMDA receptor density have been reported in cultured cells in response to *in vitro* exposure to another neurotoxicant metal, methylmercury (16). Alternatively, Zn^{+2} – a divalent metal cation like uranyl ion (UO_2^{+2}) – can cause the induction of tissue plasminogen activator (17), which results in cleavage of an NMDA receptor subunit and increased activation of the receptor channel (18). Further studies are required to discriminate which of these changes (or others) are involved in the DU-induced increased access to the receptor channel, or whether this up-regulation is due to a compensatory response to presynaptic decreases in transmitter release.

The administration of memantine, riluzole, or their combination eliminated the differences in MK-801 binding between control and DU-exposed groups that were present in the absence of drug treatment. However, as shown in Figure 9, these group changes were removed, not as a result of diminishing receptor binding in the DU groups, but as a result of increases in access to the NMDA receptor channel in MEM- and/or RLZ-treated *control* groups. RLZ also increased NMDA receptor binding in the DU-exposed groups, but otherwise exposed groups exhibited no sensitivity to changes in binding activity as a result of drug treatment. These drug effects do not have therapeutic value, since significant elevations in NMDA receptor activation are not beneficial and would be expected to increase the risk of CNS diseases such as epilepsy or those thought to possess an excitotoxic component (Huntington's, amyotrophic lateral sclerosis). Thus, chronic treatment with MEM and/or RLZ does not offer useful clinical approaches for the treatment of DU-induced neurotoxicity.

Some difficulties were encountered with the surgical implantation and function of the osmotic minipumps responsible for therapeutic drug administration to the animals in the project. These problems consisted of the development of infections at the subcutaneous minipump insertion sites and in a few instances rejection of the pumps. Accordingly, a number of experimental animals had to be replaced in the studies to insure that reliable data were obtained, and this was a significant factor in the delays in completion of the project. Difficulties in replacing departed laboratory staff delayed progress in the project to a lesser degree.

Unbudgeted expenses posed continuing problems throughout the project. These charges were primarily traceable to the higher costs of DU pellets than in previous work, the high expense of the osmotic minipumps, which could not be refilled over the period of drug administration but had to be replaced once, and the unbudgeted charges for having plasma drug levels determined by an analytical laboratory. The latter analyses were critical to establish the plasma drug levels achieved and to validate the drug administration protocols, though they were not specified in the Statement of Work and the associated expenses had not been budgeted. The tissue analyses for DU as part of Task 2 (cost

of ~$10K) were not performed due the lack of funds to cover these expenses and because these determinations were assigned a lower priority since identical analyses had been performed in an earlier USAMRMC award (see Appendix II). Otherwise, all aspects of the Statement of Work were completed by the end of the no cost extension project period.

KEY RESEARCH ACCOMPLISHMENTS

Considerable effort was invested to optimize the surgical procedures for DU pellet and osmotic minipump implants, particularly since the latter had to be replaced once in each animal at the 8 month exposure interval. The consistency and reliability of the drug administration regimen was critically important in order to demonstrate a neuroprotective/antioxidant effect for memantine and/or riluzole, and plasma drug determinations supported the other neurochemical tests being conducted.

Major scientific findings of the project included: 1) the absence of depolarization-evoked increases in hippocampal extracellular glutamate in DU groups after a shorter 9-month period of chronic exposure; 2) modest DU-induced increases in cerebellar lipid peroxidation in the absence of changes in cerebellar or striatal redox enzymes; 3) substantial DU-related increases in access to the NMDA receptor channel in frontal cortex, replicating previous increases in binding activity observed in hippocampus and sensorimotor cortex; and 4) failure of MEM and/or RLZ to diminish or eliminate DU-induced neurotoxicity in a therapeutically useful manner.

REPORTABLE OUTCOMES

Preliminary reports of the work performed on Task 1 have been reported in abstract form at the 2009 Military Health Research Forum (August 31 - September 3) and 2009 Society for Neuroscience (October 17-21) meetings. The work performed on Tasks 3 and 4 will be presented at the 2012 Society of Toxicology meeting in March. This abstract is provided below as Appendix I material. It is anticipated that two manuscripts and a review paper will result from this project in the near future.

CONCLUSIONS

Summaries of progress on the project and its importance as a scientific product are included in the preceding sections **Body** and also in **Key Research Accomplishments**.

In conclusion, the most robust component of DU-induced neurotoxicity is the striking up-regulation in brain regional MK-801 binding now seen in hippocampus and two areas of cortex. Since evidence was obtained for only a modest level of DU-related lipid peroxidation and no other signs of oxidative stress were present, there is little reason to attribute the increases in receptor binding to cellular oxidant effects. Plausible mechanisms for the changes in binding have been proposed above, but better definition of these effects awaits further investigation. It is also apparent that a 9-month DU exposure duration does not permit full development of all the neurotoxicity observable after an exposure regimen of 15-17 months – stimulated increases in extracellular glutamate were not evident, and thus there was no evidence of mitochondrial or glutamate transporter dysfunction as initially hypothesized. Finally, chronic administration of MEM and/or RLZ may have affected lipid peroxidation and ^{3}H-MK-801 binding in an understandable manner, but there was nothing in their effects that suggested clinical

value as therapeutic approaches to treat DU neurotoxicity. It appears that the effects of DU produced in the first 7 months of exposure will be difficult to reverse in any straightforward manner.

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APPENDIX I

MEMANTINE AND RILUZOLE DO NOT REVERSE THE NEUROTOXIC EFFECTS RESULTING FROM CHRONIC EXPOSURE TO DEPLETED URANIUM. S.M. Lasley, B. Hanerhoff, and K. Pastucha, Dept. of Cancer Biol. & Pharmacology, U. of Illinois Coll. of Med., Peoria, IL.

Continuing concern exists for veterans retaining fragments of depleted uranium (DU) shrapnel from Gulf War I and the resulting CNS effects. Earlier work demonstrated that rats exposed to DU for 15-17 months exhibited increases in evoked extracellular glutamate and NMDA receptor density, and suggested one basis as increased production of O_2 radicals. The goal of this study was to determine if currently prescribed therapeutic drugs can reverse these actions of chronic exposure in vivo, and to

assess various markers of oxidative stress. Male Sprague-Dawley rats had 0, 300, or 600 mg of DU pellets implanted intramuscularly at 70 days of age. After 7 months exposure osmotic minipumps were inserted subcutaneously so as to deliver memantine, riluzole, or the combination for two months during continuing DU exposure. Animals were sacrificed after 9 months exposure and the brains harvested. MK-801 receptor binding in frontal cortex was increased 83-96% by DU exposure in agreement with previous observations in hippocampus and parietal cortex; memantine and/or riluzole did not eliminate these changes but caused significant NMDA receptor up-regulation in non-exposed control animals. Cerebellar 8-isoprostane levels exhibited small increases in DU-exposed rats not receiving drugs, but while memantine reduced concentrations of this lipid peroxidation marker in all groups, it did not reverse the DU effect. This duration of DU exposure did not produce changes in cortical glutamate uptake or in striatal glutathione peroxidase or cerebellar catalase activities. These findings indicate that the most robust effect of chronic DU is increased access to the NMDA receptor ion channel. While the basis for this observation is unknown, it is associated only with modest lipid peroxidation and thus is largely independent of DU-induced oxidative stress. Furthermore, memantine and/or riluzole were ineffective in attenuating or reversing DU effects established prior to drug administration. (Supported by USAMRMC W81XWH-07-1-0468)

APPENDIX II

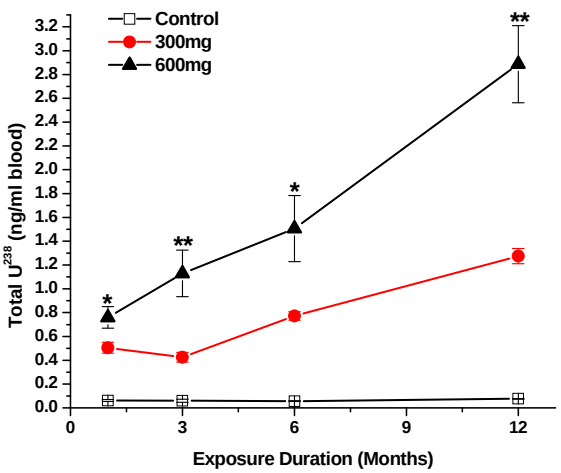

Figure A. Concentrations of uranium in rat whole blood as a function of amount and duration of exposure to implanted DU pellets as determined by ICP-MS. Values are expressed as mean ± SEM with N = 6 for each exposure group and duration. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ compared to 300 mg values at the same exposure duration.

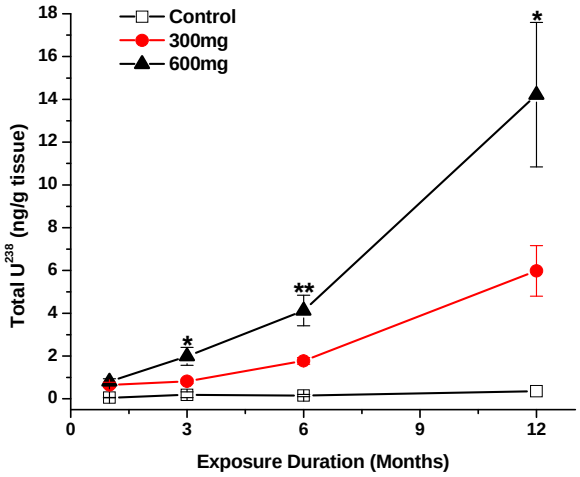

Figure B. Concentrations of uranium in rat hippocampus as a function of amount and duration of exposure to implanted DU pellets as determined by ICP-MS. Values are expressed as mean ± SEM with N = 5-6 for each exposed group and duration. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ compared to 300 mg values.

SUPPORTING DATA

None